

competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or  $^{125}\text{I}$ , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982).

Anti-PRO364 antibodies also are useful for the affinity purification of PRO364 polypeptides from recombinant cell culture or natural sources. In this process, the antibodies against a PRO364 polypeptide are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO364 polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO364 polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO364 polypeptide from the antibody.

#### H. Articles of manufacture

An article of manufacture such as a kit containing PRO364 polypeptide or antibodies thereof useful for the

diagnosis or treatment of the disorders described herein comprises at least a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition that is effective for diagnosing or treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the PRO364 or an antibody thereto. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. The article of manufacture may also comprise a second or third container with another active agent as described above.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

#### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers

is the American Type Culture Collection, Manassas, Virginia.

EXAMPLE 1: Isolation of cDNA Clones Encoding Human  
PRO364

5 An expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (Incyte EST No. 3003460) was identified that showed homology to members of the tumor  
10 necrosis factor receptor (TNFR) family of polypeptides.

A consensus DNA sequence was then assembled relative to the Incyte 3003460 EST and other EST sequences using repeated cycles of BLAST (Altshul et al., Methods in Enzymology 266:460-480 (1996)) and  
15 "phrap" (Phil Green, University of Washington, Seattle, <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>) . This consensus sequence is herein designated "<consen01>" in Figures 3A-C. The "<consen01>" consensus sequence shown in Figures 3A-C is also herein designated  
20 as "DNA44825" (see Figure 4).

Based upon the DNA44825 and "<consen1>" consensus sequences shown in Figures 3-4, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as  
25 probes to isolate a clone of the full-length coding sequence for PRO364. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in  
30 length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al.,  
35 Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

Pairs of PCR primers (forward and reverse) were synthesized:

- 5     forward PCR primer (44825.f1) 5'-CACAGCACGGGGCGATGGG-3'  
      (SEQ ID NO:5)
- forward PCR primer (44825.f2) 5'-GCTCTGCGTTCTGCTCTG-3'  
      (SEQ ID NO:6)
- forward PCR primer (44825.GITR.f) 5'-  
GGCACAGCACGGGGCGATGGGCGCGTTT-3' (SEQ ID NO:7)
- 10    reverse PCR primer (44825.r1) 5'-  
CTGGTCACTGCCACCTTCCTGCAC-3' (SEQ ID NO:8)
- reverse PCR primer (44825.r2) 5'-CGCTGACCCAGGCTGAG-3'  
      (SEQ ID NO:9)
- reverse PCR primer (44825.GITR.r) 5'-  
15    GAAGGTCCCCGAGGCACAGTCGATACA-3' (SEQ ID NO:10)

Additionally, synthetic oligonucleotide hybridization probes were constructed from the consensus DNA44825 sequence which had the following nucleotide sequences

- 20    hybridization probe (44825.p1)  
5'-GAGGAGTGCTGTTCCGAGTGGGACTGCATGTGTGTCCAGC-3' (SEQ ID NO:11)
- hybridization probe (44825.GITR.p)  
25    5'-AGCCTGGGTCAGCGCCCCACCGGGGTCCCGGGTGCGGCC-3' (SEQ ID NO:12)

30    In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO364 gene using the probe oligonucleotides and one of the PCR primers.

35    RNA for construction of the cDNA libraries was isolated from human bone marrow tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially

available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO364 [herein designated as UNQ319 (DNA47365-1206)] (SEQ ID NO:1) and the derived protein sequence for PRO364.

The entire nucleotide sequence of UNQ319 (DNA47365-1206) is shown in Figure 1 (SEQ ID NO:1). Clone UNQ319 (DNA47365-1206) has been deposited with ATCC and is assigned ATCC Deposit No. ATCC 209436. Clone UNQ319 (DNA47365-1206) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 121-123 [Kozak et al., *supra*] and ending at the stop codon at nucleotide positions 844-846 (Figure 1). The predicted polypeptide precursor is 241 amino acids long (Figure 2A). The full-length PRO364 protein shown in Figure 2A has an estimated molecular weight of about 26,000 daltons and a pI of about 6.34. A potential N-glycosylation site exists between amino acids 146 and 149 of the amino acid sequence shown in Figure 2A. Hydropathy analysis (not shown) suggested a Type I transmembrane typology; a putative signal sequence is from amino acids 1 to 25 and a potential transmembrane domain exists between amino acids 162 to 180 of the sequence shown in Figure 2A.

Analysis of the amino acid sequence of the full-length PRO364 polypeptide suggests that portions of it possess homology to members of the tumor necrosis factor receptor family, thereby indicating that PRO364 may be a novel member of the tumor necrosis factor receptor family. The intracellular domain of PRO364 contains a

motif (in the region of amino acids 207-214 of Figure 2A) similar to the minimal domain within the CD30 receptor shown to be required for TRAF2 binding and which is also present within TNFR2 [Lee et al., *supra*, (1996)]. There are three apparent extracellular cysteine-rich domains characteristic of the TNFR family [see, Naismith and Sprang, *Trends Biochem. Sci.*, 23:74-79 (1998)], of which the third CRD has 3 rather than the more typical 4 or 6 cysteines of the TNFR family. As compared to the mouse GITR (described below) the PRO364 amino acid sequence has 8 cysteines in CRD1 relative to 5 cysteines in CRD1 of mouse GITR, and the presence of one potential N-linked glycosylation site in the ECD as compared to 4 potential N-linked glycosylation sites in mouse GITR (see Figure 2B).

A detailed review of the putative amino acid sequence of the full-length native PRO364 polypeptide and the nucleotide sequence that encodes it evidences sequence homology with the mouse GITR (mGITR) protein reported by Nocentini et al., *Proc. Natl. Acad. Sci. USA* 94:6216-6221 (1997). It is possible, therefore, that PRO364 represents the human counterpart or ortholog to the mouse GITR protein reported by Nocentini et al. A comparison of the PRO364 polypeptide and the mGITR amino acid sequences is shown in Figure 2B.

EXAMPLE 2: Identification of a Potential Ligand  
for the PRO364 Polypeptide

A cDNA clone that encodes a novel polypeptide which may be a ligand that binds to the PRO364 polypeptide described herein was isolated as follows. Methods described in Klein et al., *Proc. Natl. Acad. Sci. USA* 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of *E. coli* as described in Klein et al.,

supra, PCR analysis was performed on single yeast colonies. This was accomplished by restreaking the original sucrose positive colony onto fresh sucrose medium to purify the positive clone. A single purified colony was then used for PCR using the following primers: 5'-TGTAACGACGGCCAGTTTCTCTCAGAGAAACAAGCAAAAC-3' (SEQ ID NO:13) and 5'-CAGGAACAGCTATGACCGAAGTGGACCAAAGGTCTATCGCTA-3' (SEQ ID NO:14). The PCR primers are bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites.

A library of cDNA fragments derived from human umbilical cord endothelial (HUVEC) cells fused to invertase was transformed into yeast and transformants were selected on SC-URA media. URA and transformants were replica plated onto sucrose medium in order to identify clones secreting invertase. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, DNA1840, was determined to contain a signal peptide coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of DNA1840. A full length plasmid library of cDNAs from human umbilical vein endothelial cells was titered and approximately 100,000 cfu were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The pools were grown overnight at 37°C with shaking (200rpm). PCR was performed on the individual cultures using primers specific to DNA1840. Agarose gel electrophoresis was performed and positive wells were identified by visualization of a band of the expected size. Individual positive clones were obtained by colony lift followed by hybridization with <sup>32</sup>P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and Southern blot analyses.

A cDNA clone was sequenced in entirety, wherein the complete sequence of the cDNA clone was designated DNA19355-1150. A nucleotide sequence of the DNA19355-1150 clone is shown in Figures 5A-B (SEQ ID NO:15).

5 Clone DNA19355-1150 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 21-23 [Kozak et al., *supra*] (Figures 5A-B). The predicted polypeptide precursor is 177 amino acids long (SEQ ID NO:16) and has a calculated

10 molecular weight of approximately 20,308 daltons. Hydropathy analysis suggests a type II transmembrane protein typology, with a putative cytoplasmic region (amino acids 1-25); transmembrane region (amino acids 26-51); and extracellular region (amino acids 52-177).

15 Two potential N-linked glycosylation sites have been identified at position 129 (Asn) and position 161 (Asn) of the sequence shown in Figures 5A-B (SEQ ID NO:15). Clone DNA19355-1150 has been deposited with ATCC on November 18, 1997 and is assigned ATCC deposit no.

20 209466. The polypeptide encoded by DNA19355-1150 is obtained or obtainable by expressing the molecule encoded by the cDNA insert of the deposited ATCC 209466 vector. Digestion of the vector with XbaI and NotI restriction enzymes will yield a 1411 bp fragment and

25 668 bp fragment.

Based upon a BLAST and FastA sequence alignment analysis (using the ALIGN computer program) of extracellular sequence, DNA19355-1150 shows amino acid sequence identity to several members of the TNF cytokine

30 family, and particularly, to human Apo-2L (19.8%), Fas/Apo1-ligand (19.0%), TNF-alpha (20.6%) and Lymphotoxin- $\alpha$  (17.5%) (see Figure 6).

Analysis of the polypeptide encoded by the DNA19355-1150 nucleotide sequence indicates that it is a

35 potential ligand for the human PRO364 polypeptide described herein.



EXAMPLE 3: Use of PRO364-encoding DNA as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO364 as a hybridization probe.

5 DNA comprising the coding sequence of full-length PRO364 (as shown in Figure 1, SEQ ID NO:1) or a fragment thereof is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO364) in human tissue cDNA libraries or  
10 human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of  
15 radiolabeled PRO364 polypeptide-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the  
20 filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO364 polypeptide can then be identified using standard techniques known in the art.

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EXAMPLE 4: Expression of PRO364 Polypeptides in *E. coli*

This example illustrates the preparation of forms of PRO364 polypeptides by recombinant expression in *E. coli*.

30 The DNA sequence encoding the full-length PRO364 (SEQ ID NO:3) or a fragment or variant thereof is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the  
35 selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for

ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO364 coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO364 polypeptide can then be purified using a metal chelating column under conditions that allow tight binding of the polypeptide.

#### EXAMPLE 5: Expression of PRO364 Polypeptides in Mammalian Cells

This example illustrates preparation of forms of PRO364 polypeptides by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO364-encoding DNA is ligated into pRK5

with selected restriction enzymes to allow insertion of the PRO364-encoding DNA using ligation methods such as described in Sambrook et al., *supra*. The resulting vector is called pRK5-PRO364.

5 In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg  
10 pRK5-PRO364 DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM  
15 NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The  
20 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture  
25 medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine and 200 µCi/ml <sup>35</sup>S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film  
30 for a selected period of time to reveal the presence of PRO364 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO364-encoding DNA  
35 may be introduced into 293 cells transiently using the dextran sulfate method described by Sompariyarac et al., *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg

pRK5-PRO364 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells  
5 are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged  
10 and filtered to remove cells and debris. The sample containing expressed PRO364 polypeptide can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO364 polypeptide can be  
15 expressed in CHO cells. The pRK5-PRO364 vector can be transfected into CHO cells using known reagents such as CaPO<sub>4</sub> or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel  
20 such as <sup>35</sup>S-methionine. After determining the presence of PRO364 polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing  
25 the expressed PRO364 polypeptide can then be concentrated and purified by any selected method.

Epitope-tagged PRO364 polypeptide may also be expressed in host CHO cells. The PRO364-encoding DNA may be subcloned out of the pRK5 vector. The subclone  
30 insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO364-encoding DNA insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for  
35 selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the

expressed poly-His tagged PRO364 polypeptide can then be concentrated and purified by any selected method, such as by Ni<sup>2+</sup>-chelate affinity chromatography.

5     EXAMPLE 6: Expression of a PRO364 Polypeptide in Yeast

The following method describes recombinant expression of PRO364 polypeptides in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO364  
10 polypeptide from the ADH2/GAPDH promoter. DNA encoding the PRO364 polypeptide of interest, a selected signal peptide and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of the PRO364  
15 polypeptide. For secretion, DNA encoding the PRO364 polypeptide can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, the yeast alpha-factor secretory signal/leader sequence, and linker sequences (if needed) for expression of the  
20 PRO364 polypeptide.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by  
25 precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO364 polypeptide can subsequently be isolated and purified by removing the yeast cells from  
30 the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing the PRO364 polypeptide may further be purified using selected column chromatography resins.

EXAMPLE 7: Expression of PRO364 Polypeptides in  
Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO364 polypeptides in Baculovirus-infected insect cells.

The PRO364-encoding DNA is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the PRO364-encoding DNA or the desired portion of the PRO364-encoding DNA (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 to 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression is performed as described by O'Reilley et al., *Baculovirus expression vectors: A laboratory Manual*, Oxford:Oxford University Press (1994).

Expressed poly-his tagged PRO364 polypeptide can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice

for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% Glycerol, pH 7.8) and filtered through a 0.45  $\mu$ m filter.

- 5 A  $\text{Ni}^{2+}$ -NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is  
10 washed to baseline  $A_{280}$  with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching  $A_{280}$   
15 baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with  $\text{Ni}^{2+}$ -NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing  
20 the eluted  $\text{His}_{10}$ -tagged PRO364 polypeptide are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO364 polypeptide can be performed using known chromatography techniques, including for instance,  
25 Protein A or protein G column chromatography.

EXAMPLE 8: Preparation of Antibodies  
that Bind PRO364 Polypeptides

This example illustrates the preparation of  
30 monoclonal antibodies which can specifically bind to PRO364 polypeptides.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include  
35 purified PRO364 polypeptide, fusion proteins containing a PRO364 polypeptide, and cells expressing recombinant PRO364 polypeptide on the cell surface. Selection of

the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO364 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO364 polypeptide antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO364 polypeptide. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO364 polypeptide. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against a PRO364 polypeptide is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO364 polypeptide monoclonal antibodies. Alternatively, the hybridoma



cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel  
5 exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

10        EXAMPLE 9: Assays to Detect Expression of  
         PRO364 mRNA in Human Cells and Tissues

Assays were conducted to examine expression of PRO364 mRNA in normal human tissues and in cancer cell lines.

15        Various human tissues and cancer cell lines (Clontech) were tested by Northern blot hybridization for detection of PRO364 transcripts, but none were detected. Using quantitative reverse-transcriptase PCR, PRO364 mRNA was detected in PBL, brain, bone marrow,  
20 spleen, thymus and lung, and at relatively lower levels, in kidney, heart, small intestine and liver tissues (see Figure 7). The relative mRNA expression levels were determined by quantitative PCR using a Taqman instrument (ABI) essentially as described in Heid et al., Genome  
25 Res., 6:986-94 (1996) using PRO364 specific primers and fluorogenic probes:

DNA47365.tm.f - CCACTGAAACCTTGGACAGA (SEQ ID NO:20)

DNA47365.tm.p - CCCAGTTCGGGTTTCTCACTGTGTTC (SEQ ID NO:21)

30        DNA47365.tm.r - ACAGCGTTGTGGGTCTTGTTTC (SEQ ID NO:22)

The authenticity of the PCR product was confirmed by Southern blot hybridization to the corresponding cDNA. Expression levels were normalized relative to small intestine tissue.

35        In a separate assay, primary human T cells (isolated from donor whole blood using a T cell enrichment column (R & D Systems)) and monocytes/macrophages (isolated from donor whole blood

by adherence to tissue culture flasks) were maintained in RPMI supplemented with 10% FBS and 2 mM glutamine. The cells were then treated for 24 hours with PHA (1 microgram/ml; Sigma), anti-CD3 antibody (1 microgram/ml; Pharmingen), LPS (1 microgram/ml; Sigma), TNF-alpha (1 microgram/ml; prepared essentially as described in Pennica et al., *Nature*, 312:724-729 (1984)), or the soluble DNA19355 ligand (5 microgram/ml; prepared as described in Example 10 below). The relative mRNA expression levels were then analyzed by the Taqman procedure described above. The expression levels were normalized relative to buffer-treated T cells.

The results are shown in Figure 8. Substantial up-regulation of PRO364 mRNA was observed in isolated peripheral blood T cells after stimulation by phytohemagglutinin (PHA) or by anti-CD3 antibody. High levels of expression were observed in isolated monocytes/macrophages and this expression was further increased by LPS. (See Figure 8).

EXAMPLE 10: Binding Specificity of DNA19355  
for the PRO364 Receptor

Assays were conducted to determine whether the DNA19355 polypeptide (described in Example 2 above) interacts and specifically binds with PRO364, which is believed to be a human ortholog of the murine GITR (mGITR) polypeptide described in Nocentini et al., *Proc. Natl. Acad. Sci.*, 94:6216-6221 (1997).

To test for binding, a soluble immunoglobulin fusion protein (immunoadhesin) which included a PRO364 extracellular domain (see amino acids 1-161 of Figure 2A) was expressed in insect cells. The PRO364 ECD was expressed as a C-terminus IgG-Fc tagged form in insect cells using Baculovirus (as described in Example 7 above).

A soluble DNA19355 polypeptide was prepared by expressing an ECD in *E. coli* cells. The DNA sequence encoding an extracellular region of the DNA19355

polypeptide (amino acids 52 to 177 of Fig. 5A-B; SEQ ID NO:16) was amplified with PCR primers containing flanking NdeI and XbaI restriction sites, respectively: forward: 5'- GAC GAC AAG CAT ATG TTA GAG ACT GCT AAG GAG CCC TG -3' (SEQ ID NO:17); reverse: 5'- TAG CAG CCG GAT CCT AGG AGA TGA ATT GGG GATT -3' (SEQ ID NO:18). The PCR product was digested and cloned into the NdeI and XbaI sites of plasmid pET19B (Novagen) downstream and in frame of a Met Gly His10 sequence followed by a 12 amino acid enterokinase cleavage site (derived from the plasmid):

Met Gly His His His His His His His His His Ser Ser Gly His Ile Asp Asp Asp Asp Lys His Met (SEQ ID NO:19).

The resulting plasmid was used to transform *E. Coli* strain JM109 (ATCC 53323) using the methods described in Sambrook et al., *supra*. Transformants were identified by PCR. Plasmid DNA was isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones were grown overnight in liquid culture medium LB supplemented with antibiotics. The overnight culture was subsequently used to inoculate a larger scale culture. The cells were grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells were harvested by centrifugation. The cell pellet obtained by the centrifugation was solubilized using a microfluidizer in a buffer containing 0.1M Tris, 0.2M NaCl, 50mM EDTA, pH 8.0. The solubilized DNA19355 protein was purified using Nickel-sepharose affinity chromatography.

The DNA19355 protein was analyzed by SDS-PAGE followed by Western blot with nickel-conjugated horseradish peroxidase followed by ECL detection (Boehringer Mannheim). Three predominant bands were detected, which corresponded in size to monomeric, homodimeric, and homotrimeric forms of the protein. It is believed based on this result that in its native

form, in the absence of SDS denaturation, the soluble DNA19355 protein is capable of forming homotrimers.

The soluble DNA19355 ECD molecule was then labeled with  $^{125}\text{I}$ , for testing its ability to interact with the PRO364 immunoadhesin. For comparison, immunoadhesin constructs were also made of the following TNF receptor family members: CD95, DR4, DR5, TNFR1, TNFR2, and Apo-3.

CD95, DR4, DR5, TNFR1, TNFR2, and Apo-3 immunoadhesins were prepared by fusing each receptor's ECD to the hinge and Fc portion of human IgG, as described previously for TNFR1 [Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991)]. The respective TNF receptor family members are described (and relevant references cited) in the Background of the Invention section.

For the co-precipitation assay, each immunoadhesin (5 microgram) was incubated with  $^{125}\text{I}$ -labeled soluble DNA19355 polypeptide (1 microgram) for 1 hour at 24°C, followed by protein A-sepharose for 30 minutes on ice. The reaction mixtures were spun down and washed several times in PBS, boiled in SDS-PAGE buffer containing 20 mM dithiothreitol and then resolved by SDS-PAGE and autoradiography.

The results are shown in Figure 9. The position of the molecular weight markers (kDa) are indicated in the figure. The PRO364-IgG bound to the radioiodinated soluble DNA19355 polypeptide. However, the PRO364-IgG did not bind to the immunoadhesin constructs of CD95, DR4, DR5, TNFR1, TNFR2, or Apo-3.

In another assay, human 293 cells were transiently transfected with full-length DNA19355 and the ability of receptor immunoadhesin constructs for PRO364, TNFR1, HVEM, and DcR1 to bind to those transfected cells was determined by FACS analysis. The 293 cells were maintained in high glucose DMEM media supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, 100 microgram/ml penicillin, and 100 microgram/ml streptomycin. The transfected cells ( $1 \times 10^5$ ) were incubated for 60 minutes at 4°C in 200 microliters 2%

FBS/PBS with 1 microgram of the respective receptor or ligand immunoadhesin. The cells were then washed with 2% FBS/PBS, stained with R-phycoerythrin-conjugated goat anti-human antibody (Jackson ImmunoResearch, West Grove, PA). Next, the cells were analyzed by FACS. To test the binding of the respective immunoadhesins to the transiently transfected cells, an expression vector (pRK5-CD4; Smith et al., *Science*, 328:1704-1707 (1987)) for CD4 was co-transfected with DNA19355 expression vector (see above). FITC-conjugated anti-CD4 (Pharmingen, San Diego, CA) was then used to identify and gate the transfected cell population in the FACS analysis.

As shown in Figure 10A, the PRO364-IgG bound specifically to the surface of cells transfected with the expression plasmid encoding the full length DNA19355. No such binding was observed for the TNFR1, HVEM or DcR1 immunoadhesins. The PRO364-IgG did not bind to the cells transfected with a control plasmid (data not shown).

The results demonstrate a specific binding interaction of the DNA19355 polypeptide with PRO364 and that the DNA19355 polypeptide does not interact with any of the other TNF receptor family members tested.

The DNA19355 polypeptide was identified in a human umbilical vein endothelial cell (HUVEC) library, and the DNA19355 polypeptide transcripts are readily detectable in HUVEC by RT-PCR (data not shown). A FACS analysis assay was conducted to examine whether specific binding of PRO364-IgG could be demonstrated with HUVEC by FACS analysis. HUVEC were purchased from Cell Systems (Kirkland, WA) and grown in a 50:50 mix of Ham's F12 and Low Glucose DMEM media containing 10% fetal bovine serum, 2 mM L-glutamine, 10 mM Hepes, and 10 ng/ml basic FGF. Cells were FACS sorted with PBS, PRO364-IgG, TNFR1-IgG or Fas-IgG as a primary antibody and goat anti-human F(ab')<sub>2</sub> conjugated to phycoerythrin (CalTag, Burlingame, CA).

It was found that PRO364-IgG specifically bound to HUVEC. (See Figure 10B). Neither the Fas-IgG nor the TNFR1-IgG exhibited specific binding to the endothelial cells.

5

EXAMPLE 11: Activation of NF- $\kappa$ B by DNA19355

An assay was conducted to determine whether DNA19355/PRO364 induces NF- $\kappa$ B activation by analyzing expression of a reporter gene driven by a promoter  
10 containing a NF- $\kappa$ B responsive element from the E-selectin gene.

Human 293 cells ( $2 \times 10^5$ ) (maintained in HG-DMEM supplemented with 10% FBS, 2 mM glutamine, 100 microgram/ml penicillin, and 100 microgram streptomycin)  
15 were transiently transfected by calcium phosphate transfection with 0.5 microgram of the firefly luciferase reporter plasmid pGL3.ELAM.tk [Yang et al., Nature, 395:284-288 (1998)] and 0.05 microgram of the Renilla luciferase reporter plasmid (as internal  
20 transfection control) (Pharmacia), as well as the indicated additional expression vectors for DNA19355 and PRO364 (described above) (0.1 microgram PRO364; 0.5 microgram for DNA19355 expression vector and other  
25 vectors referred to below), and carrier plasmid pRK5D to maintain constant DNA between transfections. After 24 hours, the transfected cells were harvested and luciferase activity was assayed as recommended by the  
30 manufacturer (Pharmacia). Activities (average of triplicate wells) were normalized for differences in transfection efficiency by dividing firefly luciferase activity by that of Renilla luciferase activity and were expressed as activity relative to that seen in the  
absence of added expression vectors.

As shown in Figure 11, overexpression of PRO364  
35 resulted in significant reporter gene activation, and the observed result was enhanced by co-expression of both DNA19355 and PRO364.

To examine potential intracellular mediators of the PRO364 polypeptide signaling, dominant negative mutants of certain intracellular signaling molecules involved in the pathways of NF-KB activation by TNF-alpha, IL-1, or LPS-Toll were tested.

The 293 cells were transiently transfected (as above) with the pGL3.ELAM.tk and expression vectors. In addition, the cells were transfected with the following mammalian expression vectors encoding dominant negative forms of MyD88-DN (aa 152-296); TRAF2-DN (aa 87-501); TRAF6-DN (aa 289-522); IRAK-DN (aa 1-96); IRAK2-DN (aa 1-96); RIP1-DN (aa 559-671); RIP2-DN; and NIK-DN [described in Cao et al., *Science*, 271:1128-1131 (1996); Malinin et al., *Nature*, 385:540-544 (1997); Muzio et al., *Science*, 278:1612-1615 (1997); Rothe et al., *Science*, 269:1424-1427 (1995); Ting et al., *EMBO J.*, 15:6189-6196 (1996); Wesche et al., *Immunity*, 7:837-847 (1997)]. Luciferase activity was expressed and determined as described above.

The results are shown in Figure 12. Co-transfection of a kinase-inactive mutant form of NIK, which acts as a dominant inhibitor of NF-KB activation by TNF-alpha (Malinin et al., *Nature*, 385:540-544 (1997)), IL-1 (Malinin et al., *supra*), and LPS-Toll (Yang et al., *Nature*, 395:284-288 (1998)), substantially blocked NF-KB activation through PRO364. A dominant negative TRAF2 (Rothe et al., *Science*, 269:1424-1427 (1995); Rothe et al., *Cell*, 78:681-692 (1994)) possessing an N-terminal deletion also attenuated NF-KB activation. In contrast, RIP1 (Stanger et al., *Cell*, 81:513-523 (1995)) and RIP2 (McCarthy et al., *J. Biol. Chem.*, 273:16968-75 (1998)) dominant negative mutants (RIP1-DN and RIP2-DN) did not block NF-KB activation through PRO364. Overexpression of dominant negative versions of several molecules involved in activation of NF-KB by IL-1 (Adachi et al., *Immunity*, 9:143-150 (1998); Burns et al., *J. Biol. Chem.*, 273:12203-12209 (1998); Cao et al., *Science*, 271:1128-1131 (1996), Muzio

et al., J. Exp. Med., 187:2097-2101 (1997)) and/or Tolls including MyD88, IRAK1 and IRAK2 and TRAF6 (Medzhitov et al., Mol. Cell., 2:253-258 (1998)) did not block PRO364 activation of NF-KB. IRAK1-DN (consisting of the N-terminal 96 amino acids of IRAK1) resulted in increased activation of NF-KB through PRO364 in contrast to similar experiments in which it substantially inhibited LPS-induced NF-KB activation (Yang et al., supra). Accordingly, it appears that DNA19355 polypeptide may activate the PRO364 receptor by engaging a pathway that involves TRAF2 and NIK, similar to the pathway that TNF-alpha engages through TNFR2.

15                   EXAMPLE 12: Assay to Determine Ability  
                    of PRO364 to Inhibit T cell AICD

An *in vitro* assay was conducted to determine the effect of PRO364 on T cell activation induced cell death (AICD), which involves function of endogenous Fas ligand (see Nagata et al., supra).

Human Jurkat T leukemia cells (ATCC) ( $2 \times 10^6$ ) were transfected by Superfect (Qiagen) with either the DNA19355 or PRO364 plasmids (as described above; 5 microgram), or both. Approximately 24 hours later, the cells were plated in culture plate wells precoated with PBS buffer or anti-CD3 antibody (Pharmingen) and incubated at 37° C and 5% CO<sub>2</sub>. After 18 hours, the cells were assayed for apoptosis by FACS analysis of annexin binding, as described previously by Marsters et al., Current Biology, supra.

The results are shown in Figure 13. Transfection of the Jurkat cells with DNA19355 or PRO364 inhibited the AICD response and co-expression of both the ligand and receptor molecules provided nearly complete protection against AICD. These results suggest that PRO364 is involved in regulating T cell survival, and thus PRO364 may modulate T cell function.



Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia USA (ATCC):

<u>Material</u>	<u>ATCC Dep. No.</u>
<u>Deposit Date</u>	
DNA47365-1206	ATCC 209436
November 7, 1997	
DNA19355-1150	ATCC 209466
November 7, 1997	

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of

the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to  
5 practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within  
10 the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting  
15 the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope  
20 of the appended claims.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>77</u> . line <u>8</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input checked="checked" type="checkbox"/></span>	
Name of depositary institution  American Type Culture Collection	
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, MD 20852 US	
Date of deposit November 7, 1997	Accession Number 209436
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: right; margin-bottom: 5px;">For receiving Office use only</div> <div style="margin-bottom: 5px;"><input type="checkbox"/> This sheet was received with the international application</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;">Authorized officer</div>	<div style="text-align: right; margin-bottom: 5px;">For International Bureau use only</div> <div style="margin-bottom: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;">Authorized officer</div>
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INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>77</u> . line <u>10</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution  American Type Culture Collection	
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, MD 20852 US	
Date of deposit November 7, 1997	Accession Number 209466
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: right; margin-bottom: 5px;">For receiving Office use only</div> <div style="margin-bottom: 5px;"><input type="checkbox"/> This sheet was received with the international application</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="text-align: right; margin-top: 5px;">Authorized officer</div>	<div style="text-align: right; margin-bottom: 5px;">For International Bureau use only</div> <div style="margin-bottom: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="text-align: right; margin-top: 5px;">Authorized officer</div>
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WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising DNA having at least 95% sequence identity to (a) a DNA molecule  
5 encoding a PRO364 polypeptide comprising the sequence of amino acid residues 1 to 241 of Figure 2A (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a).
2. The nucleic acid of Claim 1, wherein said DNA  
10 comprises the nucleotide sequence of SEQ ID NO:1 or its complement.
3. The nucleic acid of Claim 1, wherein said DNA  
15 comprises nucleotides 121-843 of the nucleotide sequence of SEQ ID NO:1.
4. An isolated nucleic acid comprising DNA having at least 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the cDNA  
20 in ATCC Deposit No. 209436 (DNA47365-1206), or (b) the complement of the DNA molecule of (a).
5. The nucleic acid of Claim 4 which comprises a DNA molecule encoding the same mature polypeptide  
25 encoded by the cDNA in ATCC Deposit No. 209436 (DNA47365-1206).
6. An isolated nucleic acid comprising DNA having at least 95% sequence identity to (a) a DNA molecule  
30 encoding a PRO364 polypeptide comprising the sequence of amino acid residues 1 to X of Figure 2A (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a), wherein X is any one of amino acid residues 157-167 of Figure 2A (SEQ ID NO:3).  
35
7. An isolated nucleic acid comprising DNA having at least 95% sequence identity to (a) a DNA molecule encoding a PRO364 polypeptide comprising the sequence of

amino acid residues 26 to 241 of Figure 2A (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a).

8. An isolated nucleic acid comprising DNA having  
5 at least 95% sequence identity to (a) a DNA molecule encoding a PRO364 polypeptide comprising the sequence of amino acid residues 26 to X of Figure 2A (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a), wherein X is any one of amino acid residues 157-167 of  
10 Figure 2 (SEQ ID NO:3).

9. An isolated nucleic acid comprising DNA from the group consisting of:

a) a DNA having at least 80% sequence  
15 identity to a DNA sequence encoding a PRO364 polypeptide comprising amino acid residues 26 to 241 of Figure 2A (SEQ ID NO:3);

b) a DNA sequence that hybridizes under stringent conditions to a DNA of a);

20 c) a DNA sequence that, due to the degeneracy of the genetic code, encodes a PRO364 polypeptide of a); and

d) DNA complementary to the DNA of a), b), or c).

25

10. A vector comprising the nucleic acid of any one of Claims 1 to 9.

11. The vector of Claim 10 operably linked to  
30 control sequences recognized by a host cell transformed with the vector.

12. A host cell comprising the vector of Claim 10.

35 13. The host cell of Claim 12, wherein said cell is a CHO cell.

14. The host cell of Claim 12, wherein said cell is an *E. coli*.

15. The host cell of Claim 12, wherein said cell is a yeast cell.

16. A process for producing a PRO364 polypeptide comprising culturing the host cell of Claim 12 under conditions suitable for expression of said PRO364 polypeptide and recovering said PRO364 polypeptide from the cell culture.

17. An isolated PRO364 polypeptide comprising amino acid residues 1 to 241 of Figure 2A (SEQ ID NO:3).

18. An isolated PRO364 polypeptide encoded by the cDNA insert of the vector deposited as ATCC Accession No. 209436 (DNA47365-1206).

19. An isolated PRO364 polypeptide comprising amino acid residues 1 to X of Figure 2A (SEQ ID NO:3), wherein X is any one of amino acid residues 157-167 of Figure 2A (SEQ ID NO:3).

20. An isolated PRO364 polypeptide comprising amino acid residues 26 to 241 of Figure 2A (SEQ ID NO:3).

21. An isolated PRO364 polypeptide comprising amino acid residues 26 to X of Figure 2A (SEQ ID NO:3), wherein X is any one of amino acid residues 157-167 of Figure 2A (SEQ ID NO:3).

22. An isolated PRO364 polypeptide comprising a polypeptide selected from the group consisting of:

a) a PRO364 polypeptide comprising amino acid residues 26 to X of Figure 2A (SEQ ID NO:3),

wherein X is any one of amino acid residues 157-167 of Figure 2A (SEQ ID NO:3); and

b) a fragment of a), wherein said fragment is a biologically active polypeptide.

5

23. A chimeric molecule comprising a PRO364 polypeptide fused to a heterologous amino acid sequence.

24. The chimeric molecule of Claim 23, wherein  
10 said heterologous amino acid sequence is an epitope tag sequence.

25. The chimeric molecule of Claim 23, wherein  
15 said heterologous amino acid sequence is a Fc region of an immunoglobulin.

26. An antibody which specifically binds to a PRO364 polypeptide.

20 27. The antibody of Claim 26, wherein said antibody is a monoclonal antibody.

28. A composition comprising an isolated PRO364 polypeptide of Claims 17, 18, 19, 20, 21, or 22 and a  
25 carrier.

29. The composition of Claim 28 wherein said carrier is a pharmaceutically-acceptable carrier.

30 30. A method of modulating apoptosis in mammalian cells, comprising exposing said cells to an effective amount of PRO364 polypeptide.

31. A method of modulating NF-KB activation in  
35 mammalian cells, comprising exposing said cells to an effective amount of PRO364 polypeptide.



32. A method of modulating a proinflammatory or autoimmune response in mammalian cells, comprising exposing said cells to an effective amount of PRO364 polypeptide.

1/15

1 CACGCACTTC ACCTGGGTG GGAATCTCAG GTCATGAACG GTCCAGGCCA CCTCGGGCA GGGCGGTGA GGACGGGGAC GGGCGGTGTC CAACGTGGCTG  
 GTGCGTGAAG TGGACCCAGC CTAAGAGTC CAGTACTGC CAGGTGCGT GGAGGCCCGT CCCGCCCTG CCCGCCACAG GTTGACCCGAC  
 101 TGGGTCTTTG AAACCCGAGC ATGCCACAGC ACGGGCGAT GGGCGCTGT GGGCCCTGT GCGGCTGTGC GCGCTCAGCC TGGGTACAGC  
 ACCCGAGAAC TTTGGGCTCG TACCGTGTG TGCCTGCTA CCCCGGCAAA GCGCGGACG CGACGACACG CCGAGTCCG ACCCAGTCGC  
 1 M A Q H G A M G A F R A L C G L A L L C A L S L G Q R  
 ^MET  
 201 CCCACCCGGG GGTCGCCGGT GGGCCCTCG GCGCTCTG GAGCGAGCG GCGCTGCTGC CGGTTCACA CGAGCGGCTG CTGCCGCGAT  
 GGGGTGGCCC CCAGGCCCA. CCGCGGACC CCGGAGGAC GAACCTGCG CTTGCTGCG CCGACGACG GCCAAGTGT GCTGGCGAC GACGGCGCTA  
 28 P T G G P G C G P G R L L L G T G T D A R C C R V H T T R C C R D  
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 ATGGCCCCC TCCTCAGAC AAGCTCACC CTGAGGTACA CACAGTCCG ACTTAAGTG AGCCTCTGG GAACGACGTG CTGACGCGC GTGTGGAA  
 61 Y P G E C C S E W D C M C V Q P E F H C G D P C C T T C R H H P C  
 401 GTCCCCCAGG CCAGGGGTA CAGTCCCAGG GGAATTCAG TTTGGCTTC CAGTGTATCG ACTGTGCTC GGGACCTTC TCCGGGGCC ACGAAGGCCA  
 CAGGGGTCC GTCCCCCAT GTCCGGTCC CTTTAAGTC AATACCGAAG GTACATAGC TGACACGAG CCCTGGAG AGGCCCCCG TGCTCCGCT  
 95 P P G Q G V Q S Q G K F S F G F Q C I D C A S G T F S G G H E G H ^47365.tm.f  
 501 CTGCAACCT TGGACAGCT GACCCAGT CGGTTCTC ACTGTGTTCC CTGGAACAA GACCCACAA GCTGTGTGG TCCAGGGTC CCCGCCGGCA  
 GACGTTTGA ACCTGTCTGA CGTGGGTCA GCCCAAGAG TGACACAAAG GACCTTGT CTGGTGTG CGACACACG AGGTCCCGG GGGCGGCGGT  
 128 C K P W T D C T Q F G F L T V F P G N K T H N A V C V P G S P P A  
 ^47365.tm.p  
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 CTCGCGAAC CCACCGACTG GCACGAGGAG GACCGGCACC GCGGACGCA GGAGGAGGAC TGGAGCCGG TCGAACCTGA CGTGTAGACC GTCGACTCCT  
 161 E P L G W L T V L L A V A A C V L L L T S A Q L G L H I W Q L R S  
 701 GTAGTGCAT GTGCCCCCGA GAGACCCAGC TGCTGTGGA GGTGCCCGG TCGACCGAG ACGCCAGAG CTGCCAGTTC CCGAGGAG AGCGGGGCGA  
 CAGTACGTA CACCGGGGT CTTGGGTG ACGAGACCT CCACGGGCG AGTGGCTTC TGGGTCTTC GACGTCAAG GGGTCTCTC TCGCCCCGCT  
 195 Q C M W P R E T Q L L L E V P P S T E D A R S C Q F P E E R G E  
 801 GCGATCGCA GAGAGNAG GCGGCTGG AGACTGTG GTGTAGCCT GCGGCTCTC CCGGCCACC GACCGCAGC AGCCCCCTCC CAGGAGCTCC  
 CGCTAGCGT CTCTCTTC -CGCGGACCC TCTGACACC CACACTCGGA CCGCAGGAG -GCCCGGTG CTGGGTGCG TCGGGGAGG GTCTCGAGG  
 228 R S A E E K G R L G D L W V O  
 901 CCAGGCCGA GGGCTCTG GTTGTCTT GGGCGGGG CTGTCTCCCT GGCAGCAGAA GTGGGTGAC GAAGGTGCA GTGACGAGC CCCTGGACCA  
 GGTCCGGCGT CCGGAGAG CAGACGAGA CCCGCCCGG GACAGGGGA CCGTCTCTT CACCCAGTC CTTCACCGT CACTGGTCCG GGGACCTGGT  
 1001 TGCAGTTC  
 ACGTCAAG

FIG. 1

2/15

<MW: 26000, pI: 6.34, NX(S/T): 1  
 <greatest homology to MMU82534\_1 glucocorticoid induced TNFR related>  
 < protein - may be human homolog of this mouse gene>  
 <25-26 potential signal peptide cleavage site>  
 <33 4 TNFr - Cys repeat domains>  
 <146 potential N-linked glycosylation site>  
 <162 start potential transmembrane domain>  
 <180 end potential transmembrane domain>  
 < 1 10 20 30 40 50 60 70  
 < | | | | | | | | | | | | | | | |  
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 < 71 80 90 100 110 120 130 140  
 < | | | | | | | | | | | | | | | |  
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 < 141 150 160 170 180 190 200 210  
 < | | | | | | | | | | | | | | | |  
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 < 211 220 230 240  
 < | | | | | | | | | | | | | | | |  
 STEDARSCQFPEEERGERSAEEKGRGLDLWV

FIG. 2A

3/15

CRD 1

1 MAQHGA MGA FRA L CGLAL LCA L LCA L S LGQRP - TGGGPGGCGPGGRLLLG TGT DARC

1 - - - - - MGA WAM L YGV SMLC V L D LGQ P S V E E P G C G P G K V Q N G S G N N T R C

CRD 2

50 CRVHTTRCCRDY PGE E C CSEW D C M CVQ PEFFHCGDPC C T TCRHHPCC PPGQGR

45 CSLYA - - - - - PGKE DCPKER C I C V T P E Y H C G D P C C K T C K H Y P C Q P G Q R

CRD 3

100 VQSQ GKFS FGFQ C I D C ASGTFS G GHE G HC K P W T D C T Q F G F L T V F P G N K T H

88 VESQGD I V F G F R C V A C A M G T F S A G R D G H C R L W T N C S Q F G F L T M F P G N K T H

T M

150 NAVC VPGSSP PAE PLG W L T V V L L A V A A C V L L L T T S A Q L G L H I W Q L R S Q C M W P

138 NAVC IPEPLPTE Q Y G H L T V I F L V M A A C I F F L T T V Q L G L H I W Q L R R Q H M C P

200 RETQ L L L E V P P S T E D A R S C Q F P E E E R G E R S A E E K G R L G D L W V

188 RETQ P F A E V Q L S A E D A C S F Q F P E E E R G E Q - T E E K C H L G G R W P

PRO364  
mGTR

PRO364  
mGTR

PRO364  
mGTR

PRO364  
mGTR

PRO364  
mGTR

**FIG. 2B**

4 / 15

<consen01> 1 GGCACAGCACGGGGCGATGGGCGCGTTTCGGGGCCCTGTGCGGCCTGGCGC  
<consen01> 51 TGCTGTGCGCGCTCAGCCTGGGTACGCGCCCCACCGGGGT-CCCGGGTG  
<consen01> 101 CGGCCCTGGGCGCCTCCTGCTTGGGACGGGAACGGACGCGCGCTGCTGCC  
<consen01> 151 GGGTTCACACGACGCGCTGCTGCCGCGATTACCCGGGCGAGGAGTGCTGT  
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<consen01> 251 TTGCTGCACGACCTGCCGGCACCACCCTTGTCCCCCAGGCCAGGGGGTAC  
<consen01> 301 AGTCCCAGGGGAAATTCAGTTTTGGCTTCCAGTGATCGACTGTGCCTCG  
<consen01> 351 GG-GACCTTCTCCGGGGGCCACGAAG--GCCACTGCAAACCTTGGACAGA  
<consen01> 401 CTGCACCCAGTT-CGGG-TTCTCACTGTGTTCCCTGGGGAACAAGACCC  
<consen01> 451 -ACAA-CGCTGTGTGCGTCCCAGGGTCCCCG-CCGGCAGAGCCGCTT-GG  
<consen01> 501 GTGGCTGACCGTCGTCCTCCTGGCCGT-GGCCGCTGCGTC-TCCTCCTG  
<consen01> 551 ACCTCGGCCCAGCTTGGACTGCACATCTGGCAGCTGAGGAGTCAGTGCAT  
<consen01> 601 GTGGCCCCGAGGTCTGTACAGCCTGGTGCGGGGAGGTGGGAGCATGGCT  
<consen01> 651 GCCTGCTGACCGTGGCCCCCCTGCATAGACCCAGCTGCTGCTGGAGGTGC  
<consen01> 701 CGCCGTCGACCGAAGACGCCAGAAGCTGCCAGTTCCCCGAGGAAGAGCGG  
<consen01> 751 GGCGAGCGATCGGCAGAGGAGAAGGGGCGGCTGGGAGACCTGTGGGTGTG  
<consen01> 801 AGCCTGGCTGTCTCCGGGGCCACCGACCGCAGCCAGCCCCTCCCCAGGA  
<consen01> 851 GCTCCCCAGGCCGCGAGGGGCTCTGCGTTCTGCTCTGGGCCGGGCCCTGCT  
<consen01> 901 CCCCTGGCAGCAGAAGTGGGTGCAGGAAGGTGGCAGTGACCAGCGCCCTG  
<consen01> 951 GACCATGCAGTTC

FIG. 3

SUBSTITUTE SHEET (RULE 26)

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1 GGCACAGCAC GGGGGATGG GCGGTTTCG GGCCTGTGTC GGCCTGGGCG TGCTGTGGCG GCTACGCTG GGTACGGCC CCACCGGGGG TCCCGGTGTC  
 CCGTGTGTCG CCGCGTACC CCGCAAGC CCGGACACG CCGGACCGG ACACACGCG CGAGTCGGAC CAGTCGCG GGTGGCCCC AGGGCCCCACG  
 1 M G A F R A L C G L A L L C A L S L G Q R P T G G P G C  
 ^44825.GITR.f ^orf  
 ^44825.f1

101 GGCCTGGGC GCCTCTGCT TGGACGGGA ACAGGACGGC GCTGCTGGC GGTTCACAG ACAGGCTGCT GCGCGATT CCGGGGAG GAGTGTGTT  
 CCGGACCGG CCGAGGACGA ACCCTGCCCT TGCTGCGCG CGAGGACGGC CCAAGTGTG TGCGGACGA CGGCGCTAT GGGCCCCGTC CTCACGACAA  
 29 G P G R L L L G T G T D A R C C R V H T T R C C R D Y P G E C C S  
 ^44825.GITR.p ^44825.r2

201 CCGAGTGGGA CTGCAIGTGT GTCCAGCCTG AATTCCACTG CGAGACCCCT TGCTGCAGCA CTGCGGGCA CCACCTTGT CCCCCAGCC AGGGGTACAA  
 GGCTACCCCT GACGTACACA CAGGTGCGAC TTAAGGTGAC GCCTCTGGA ACAGGCTGCT GGACGGCCGT GGTGGGAACA GGGGGTCCGG TCCCCCATGT  
 63 E W D C M C V Q P E F H C G D P C C T T C R H H P C P P G Q G V Q

301 GTCCAGGGG AATTTCAGTT TTGGTTCCA GTGATCGAC TGTGCTCGG GGACCTTCTC CGGGGGCCAC GAAGGCCACT GCAACCTTG GACAGACTGC  
 CAGGGTCCCC TTAAAGTCAA AACCGAGGT CACATAGTGT ACAGGAGCC CTGGAAGAG GCCCGCGTG CTTCCGTTGA CGTTTGAAC CTGCTGACG  
 96 S Q G K F S F G F Q C I D C A S G T F S G G H E G H C K P W T D C  
 ^44825.GITR.r

401 ACCAGTTGC GGTTCCTAC TGTGTTCCCT GGGCAACAAG ACCACACAG CTGTGTGCT CCCAGGTC CCCCCGGCAG AGCCGCTTG GTGGTGTACC  
 TGGTCAAGC CCAAGAGTG ACACAGGGA CCCCTTGTT TGGGTGTGTC GACACAGCA GGTGCCCGTC GGGGGCCGTC TCGGGAAC CACCGACTGG  
 129 T Q F G F L T V F P G E Q D P Q R C V R P R V P A G R A A W V A D R

501 GTGCTCTCC TGCCGTTGG CGCTGCGTC TCCTCTGAC CTGCGGCCAG CTGACTGC ACATCTGGA GCTGAGGAGT CAGTGCATGT GGGCCCGAGG  
 CAGCAGGAGG ACGGGACCG GCGGAGCGAG AGGAGGACTG GAGCCGGTC GAACCTGAC TGTAGACCGT CGACTCTCA GTCACGTACA CCGGGCTCC  
 163 R P P G R G R L R L L L T S A Q L G L H I W Q L R S Q C M W P R G

601 TGTGTCACAG CCTGGTGGG GGAGTGGGA GCATGGCTG CTGCTGACG TGCCCCCCT GCATAGACCC AGCTGCTGCT GGAGTGCCG CCGTCGACCG  
 AGACAGTGTG GGACACGCC CCTCCACCCT CGTACCGAG GAGGACTGCG ACCTGGGGA CGTATCTGG TCACGACGA COTCCACGGC GGCAGCTGGC  
 196 L S Q P G A G R W E H G C L L T V A P L H R P S C C W R C R R P

701 AAGACGCCAG AAGTGCAG TTCCCGGAG AAGAGCGGG CGAGCGATCG GCAGAGAGA AGGGCGGCT GGGAGACCTG TGGGTGTGAG CCTGGCTGTC  
 TTCTGGGTC TTCGACGTC AAGGGCTCC TTCTCGCCCC GCTCGCTAGC CGTCTCTCT TCCCGCCGA CCTCTGAC ACCCAGCTC GGACCGACAG  
 229 K T P E A A S S P R K S G A S D R Q R R R G G W E T C G C E P G C P

801 CTCGGGGCC ACCGACGCA GCCAGCCCT CCCCAGGAG TCCCGAGCC GCAGGGCTC TGCTTCTGCT TGTGGGGCGG GCTCTGCTC CCTGGCAGCA  
 GAGGCCCGG TGGTGGCGT CCGTGGGGA GGGTCTCTG AGGGTCTCG AGGGTCTCG CGTCCCGAG ACAGAGAGG AGACCGGCC CCGGACGAGG GGACGCTCGT  
 263 P G P P T A A S P S P G A P Q A A G A L R S A L G R A L L P W Q Q  
 ^44825.f2

901 GAAGTGGTG CAGGAGGTG GCAGTGACCA GCGCCCTGGA CCATGCACTT CCGNGGCCGG GTNGGCCCT  
 CTTACCCAC GTCTTCCAC CGTCACTGT CGGGGACCT GTTACGTCAA GCCNCGGCC CACCGGGA  
 296 K W V Q E G G S D Q R P G P C S S X A G X A  
 ^44825.r1

FIG. 4

SUBSTITUTE SHEET (RULE 26)

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1 CAGCTCTCAT TTCTCCAAA ATGTGTTTGA GCCACTTGA AATATGCGT TTAAGCCATT CAAGAACTCA AGGAGCTCAG AGATCATCTT GGAAGCTGTG  
 1 GTGAGAGTA AAGAGGTTTT TACACAACT CGGTGAACCT TTATACGGA AATTCGGTAA GTTCTTGAGT TCCTCGAGTC TCTAGTAGGA CCTTCGACAC  
 MetCysLeuS erHisLeuGl uAsnMetPro LeuSerHisS erArgThrGl nGlyAlaGln ArgSerSerT rplysLeuTrp  
 101 GCTCTTTTGG TCAATAGTTA TGTGCTANT TCITGCTCC TTCAGTTGGC TAATCTTTAT TTTTCTCAA TTAGAGACTG CTAAGGAGCC CTGATGGCT  
 CGAGAAACG AGTTATCAAT ACAACGATAA AGAACGAGG AAGTCAACCG ATTGAATA AAAAGAGGTT AATCTCTGAC GATTCTCTGG GACATACCGA  
 28 LeuPheCys SerIleValM etLeuLeuPh eLeuCysSer PheSerTrpL euillePheIl ePheLeuGln LeuGluthrA laLysGluPr oCysMetAla  
 201 AAGTTTGGAC CATTACCCTC AAAATGGCAA ATGGCATCTT CTGAACCTCC TTGCGTGAAT AAGGTGCTG ACTGGAAGCT GGAGATACTT CAGAATGGCT  
 TTCAAACCTG GTATGGGAG TTTTACCGTT TACCGTAGAA GACTTGGAGG AACGCACCTA TTCCACAGAC TGACCTTCGA CCTCTATGAA GTCTTACCGA  
 61 LysPheGlyP roLeuProSe rLysTrpGln MetAlaSerS erGluProPr oCysValAsn LysValSerA spTrpLysLe uGluIleLeu GlnAsnGlyLeu  
 301 TATATTTAAT TTATGGCCAA GTGGCTCCCA ATGCAAACTA CAATGATGTA GCTCCTTTTG AGTGCGGCT GTATAAAAAC AAAGACATGA TACAAACTCT  
 ATATAAATTA AATACCGGTT CACCGAGGTT TACGTTTGTAT GTTACTACAT CGAGGAAAC TCCACCCCGA CATATTTTGG TTTCTGTACT ATGTTTGA  
 95 TyrLeuIl eTyrGlyGln ValAlaProA snAlaAsnTy rAsnAspVal AlaProPheG luValArgLe uTyrLysAsn LysAspMetI leGlnThrLeu  
 401 AACAAACAA TCTAAATCC AAAATGTAGG AGGACTTAT GAATGTCATG TTGGGGACAC CATAGACTTG ATATCAACT CTGAGCATCA GGTCTTAAAA  
 TTGTTTGTGT AGATTTTAGG TTTTACATCC TCCTGAATA CTTAACGTAC AACCCCTGTG GTATCTGAAC TATAAGTTGA GACTCGTAGT CCAAGATTTT  
 128 ThrAsnLys SerLysIleG lnAsnValGl yGlyThrTy rGluLeuHisv alGlyAsph rIleAspLeu IlePheAsnS erGluHisGl nValLeuLys  
 501 AATAATACAT ACTGGGGTAT CATTTTACTA GCAATCCCTC AATTCATCTC CTAGAGACTT GATTGATCT CCTCATTCCTC TTCAGCACAT GTAGAGGTGC  
 TTATTATGTA TGACCCCATTA GTAAATGAT CGTTTAGGGG TTAAGTAGAG GATCTCTGAA CTAAACTAGA GGAGTAAGGG AAGTCGTGTA CATCTCCACG  
 161 AsnAsnThrT yrTrpGlyIl eIleLeuLeu AlaAsnProG lnPheIleSe rAM\*  
 601 CAGTGGGTGG ATTGGAGGGA GAAGATATTC AATTTCTAGA GTTTGTCTGT CTACAAAAT CAACACAAAC AGAACTCCTC TGCACGTGAA TTTTCATCTA  
 GTCACCCACC TAACCTCCCT CTTCTATAAG TTAAGATCT CAACAGACA GATGTTTTTA GTTGTGTTTG TCTTGAGGAG ACGTGCACCT AAAAGTAGAT  
 701 TCATGCCTAT CTGAAGAGA CTCAGGGGAA GAGCCAAAGA CTTTGGTGTG GATCTGCAGA AATACTTTCAT TAATCCATGA TAAACAAAT ATGGATGACA  
 AGTACGGATA GACTTTCTCT GAGTCCCTT CTCGGTTTCT GAAAACCAAC CTAGACGCTT TTATGAAGTA ATTAGTACT ATTTTGTITA TACCTACTGT  
 801 GAGGACATGT GCTTTTCAA GAATCTTTAT CTAATTTCTG AATTCATGAG TGA AAAATG GAGTTCTATT CCCATGGAAG ATTTACCTGG TATGCAAAAA  
 CTCCTGTACA CGAAAGTTT CTTAGAAATA GATTAGAAC TTAAGTACTC ACCTTTTAC CTCAGATAA GGTACCTTC TAAATGGACC ATACGTTTTT  
 901 GGATCTGGGG CAGTAGCCTG GCTTTGTCTT CATATTTCTT ATTCATCTT CTCATACCTC CATCTTCTGA GACCTCTCCA ATAAAAAGTA  
 CCTAGACCCC GTCATCGGAC CGAAACAAGA GTATAAGAAC CCGACGACAT TAAGTAAGAA GAGTATGAGG GTAGAAGACT CTGGAGGGT TATTTTTCAT  
 1001 GACTGATAGG ATGGCCACAG ATATGCCTAC CATACCTTAC TTTAGATATG GTGGTGTAG AAGATAAAGA ACAATCTGAG AACTATTGGA ATAGAGGTAC  
 CTGACTATCC TACCGGTGTC TATACGGATG GTATGGGATG AAATCTATAC CACCACAATC TTCTATTCT TGTTAGACTC TTGATAACCT TATCTCCATG

FIG. 5A

SUBSTITUTE SHEET (RULE 26)

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1101 AAGTGGCATA AAATGGAATG TAGCTATCT GGAATTTCT CTTGCTTTTA TCTTCTCAG GATGCAGGT GCTTTAATAA GCCTATACAA AGGAGTCATT  
TTACCGTAT TTTACCTTAC ATGGGATAGA CTTTAAAGA GAACCAAAAT AGAAGGAGTC CTACGTCCCA CGAAATTTT CCGRATAGTT TCCTCAGTAA

1201 CCGAACCCCTC ACGTAGAGCT TTGTGAGACC TTACTGTGTC TGTGTGTGC TAACATGTC TAATTGTAAA GAAAGAGTAA CCATTAGTAA TCATTAGGTT  
GGCTTGGGAG TGCATCTCGA AACACTCTGG AATGACRACC ACACACACAG ATTTGTAACG ATTAACATTT CTTTCTCATT GGTAATCAT AGTAATCCAA

1301 TAACCCACAGA ATGGTATTAT CATTAAGTGA TTATGTCATG TAATGATTTA GTATTTTAG CTAGCTTTCC ACAGTTTGA AAGTCTTTT GTAAAACRGT  
ATTGGGTCT TACCATAATA GTAATGACCT AATACAGTAC ATTACTAAAT CATAAAATC GATCGAAAGG TGTCARAGT TTCACGAAAG CATTTTGTCA

1401 TAGCAATCT ATGAAGTTAA TTGGGCAGGC ATTTGGGGGA AAATTTTAGT GATGAGRATG TGTAGCATA GCATAGCCAA CTTTCTCTCAA CTCATAGGAC  
ATCGTTAAGA TACTTCAANT AACCCGTCCG TAAACCCCTT TTTAAAATCA CTACTCTTAC ACTATCGTAT CGTATCGTT GAAAGGAGTT GAGTATCCTG

1501 AAGTGACTAC AAGAGGCAAT GGTAGTCCC CTGCATTGCA CTGTCTCAGC TTTAGRATG TTTATTTCTGC TATCGTGTTA TAAGACTCTA AAACCTTAGCG  
TTCACTGATG TTCTCCGTTA CCCATCAGG GACGTACGT GACAGAGTCG AAATCTTAAC AATAAGACG ATAGCACAAT ATTCTGAGAT TTTGAATCGC

1601 AATTCACTTT TCAGGAAGCA TATTTCCCTT TAGCCCAAGG TGACAGAGT GAAGCTACAA CAGATCTTT CTTTACCAGC ACATTTTTT TTTTTTTTCC  
TTAAGTGAAA AGTCCTTCGT ATAGGGGGA ATCGGGTTCC ACTCGTCTCA CTTGATGTT GTCTAGAAAG GAAATGTCG TGTGAAAAA AAAAAAAGG

1701 TGCCTGAATC AGGGAGATCC AGGATGCTGT TCAGGCCAAA TCCCAACCAA ATTCCCTTT TCACTTTGA GGGCCCATCT TAGTCAAATG TGCTAACTTC  
ACGGACTTAG TCCCTCTAGG TCCTACGACA AGTCGGGTTT AGGTTGGTT TAAGGGGAAA AGTGAAACGT CCGGGGTAGA ATCAGTTTAC ACGATTGAAG

1801 TAAAATAATA AATAGCACTA ATTCAAAATT TTTGGAATCT TAATTAGCT ACTTGCNGGT TCGTTGTTGA AAGGNATATA ATGATTACAT TGTAAACAAA  
ATTTTATTAT TTATCGTGAT TAAGTTTAA AAACCTTGA ATTTAATCGA TGAACGNCCA ACGAACAACT TTCCNTATAT TACTAATGTA ACATTTGTTT

1901 TTTAAATAT TTATGGATAT TTGTGAAAAG CTGCATTATG TTAATAATA TTACATGTAA AGCT  
AAATTTTATA AATACCTATA AACACTTTTC GACGTAATAC AATTATTAT AATGTACATT TCGA

FIG. 5B



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		A		A'		B'			
DNA	19355	52	ETAKEPCMAKFG	-----	PLPSK	-----	WQMASSEP-PCVNVKVS <del>DK</del> ---		
	TNF- $\alpha$	84	PSDK-PVAHVVA	-----	NQAEG-QLQ	-----	WLNRR-ANALLANGVELRDNQ		
	Apo2L	119	GPQR-VAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLH-LRNGE						
	CD95L	142	E-LR-KVAHLTG	-----	KNSRSM-PL	-----	WEDTY-GIVLLS-GVKYKGG		
	LT $\alpha$	59	STLK-PAHLIG	-----	DPSKQN-SLL	-----	WRANT-DRAFLQDGFSLSNNS		
		B		C		D		E	
DNA	19355	86	LEILQGLYLIYGVAPNAN	-----	YNDVAPFEVRLYKNK-DMIQTLTNK-SKIQN				
	TNF- $\alpha$	124	LVPSEGLYLIYSQVLFKGGCP	-----	STHVLLTHTISRIVS	-----	YQTKVNLLSAIKS		
	Apo2L	175	LVIHEKGFYIYSQTYFRFQEEIKENTKNDKQMVQYIYKTSYDPPI--LLMKSARNSC						
	CD95L	182	LVINETGLYFVYSKVYFRGQSC	-----	NNLPLSHKVYMRNSKY--PQDLVMMEGKMMS				
	LT $\alpha$	99	LLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPFHVPLL-SSQKMVY						
		F		G		H			
DNA	19355	136	-----	VGGTYELHVGDTIDLIFNSEHQVLKNNT-YWGIILLANPQF-IS					
	TNF- $\alpha$	176	PCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL						
	Apo2L	232	-----	WSKDAEYGLYSITQCGIFELKENDRI FVSVTNEHLIDMDHEA-SFFGAFLVG					
	CD95L	233	-----	YCTTGQMWARSYLGAVFNLTSAHLVYVNVSELSLVNF-EESQTFFGLYKL					
	LT $\alpha$	157	-----	PGLQEPWLHSMYHGAAAFQLTQGDQLSTHTDGIPLHLV-SPSTVFFGAFAL					

FIG. 6

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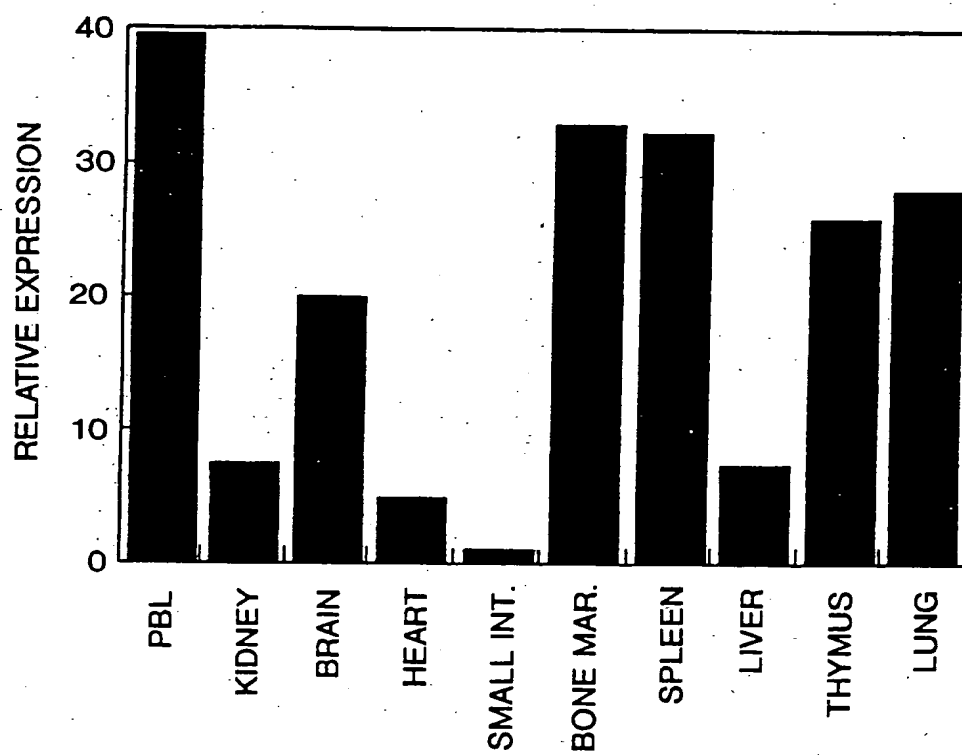


FIG. 7

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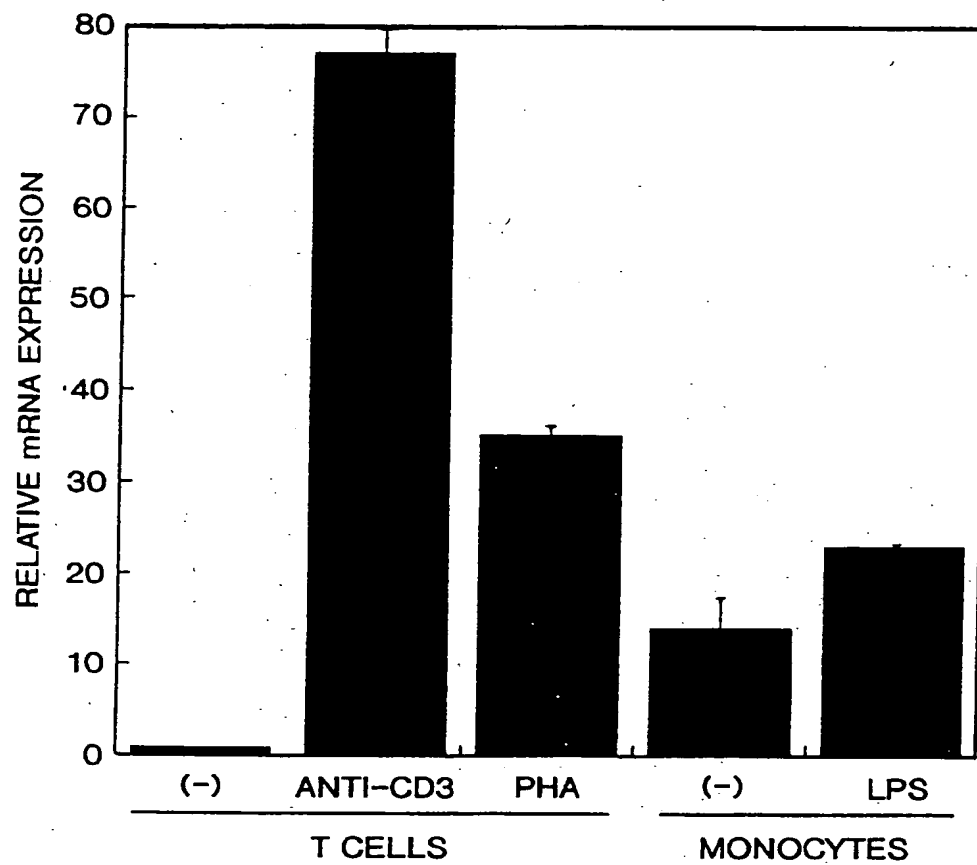


FIG. 8

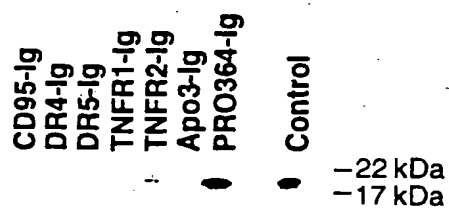


FIG. 9

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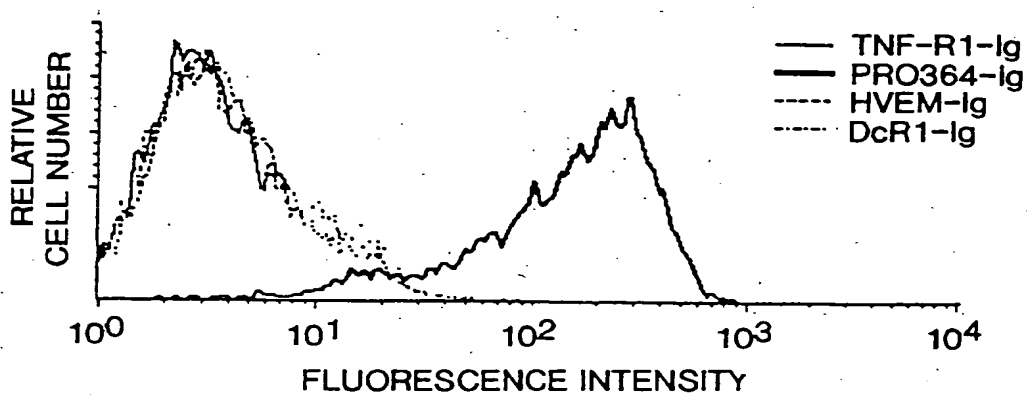


FIG. 10A

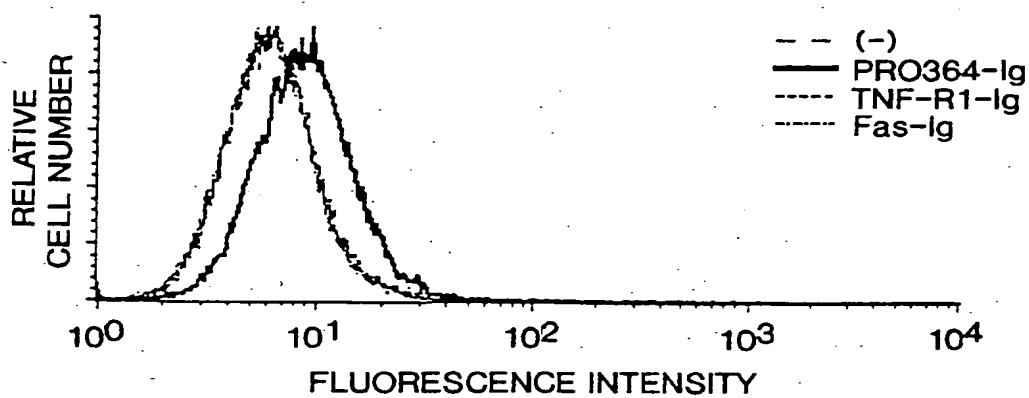


FIG. 10B

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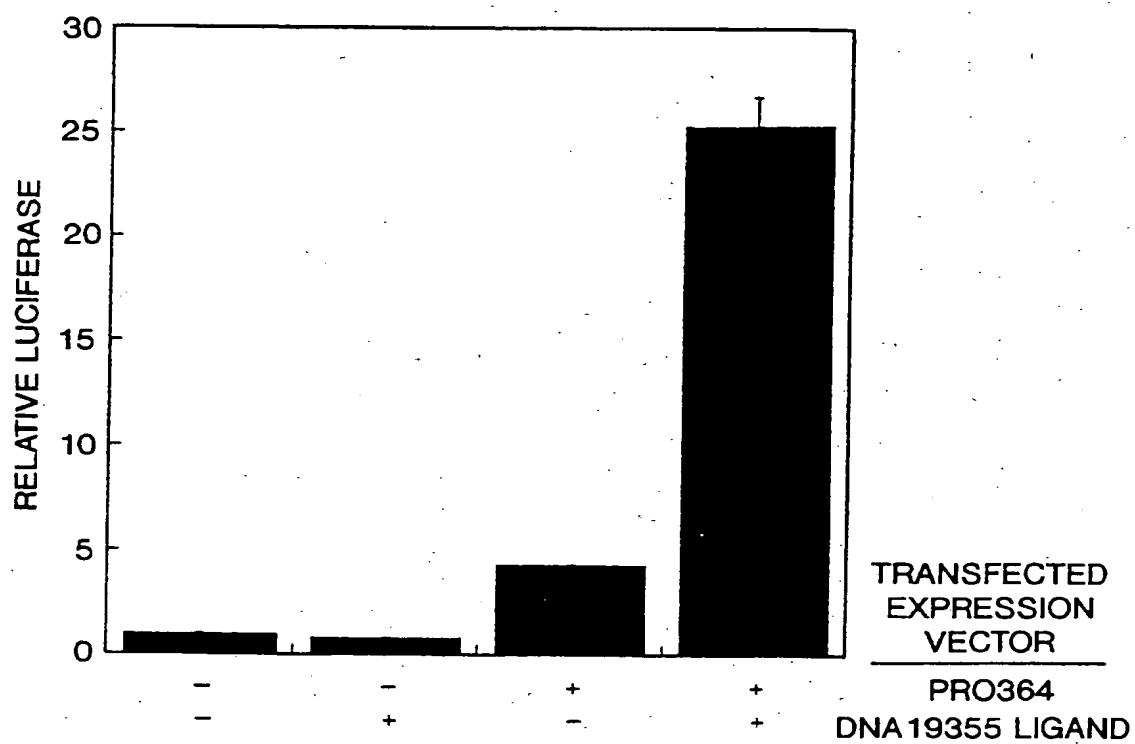


FIG. 11

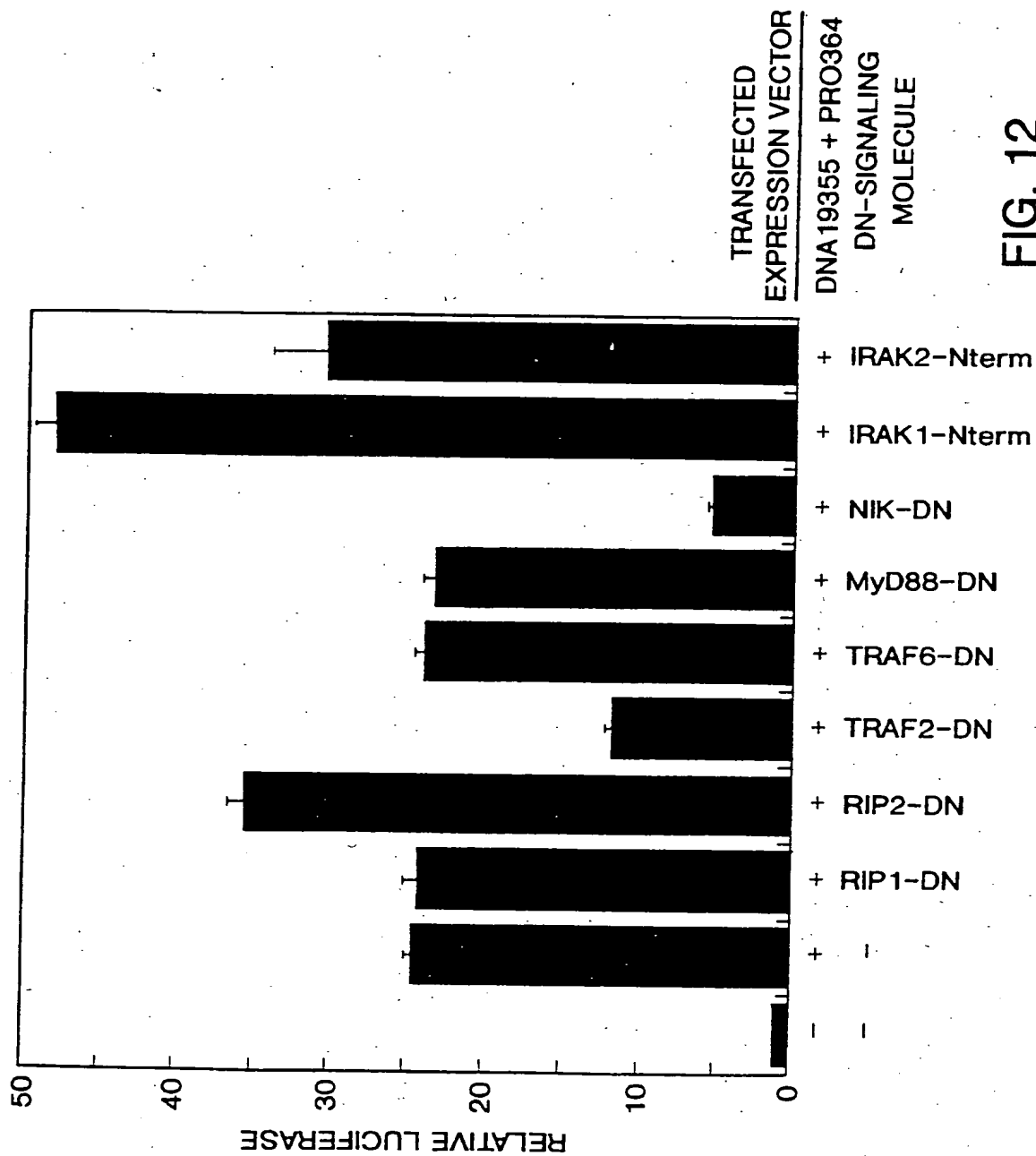


FIG. 12

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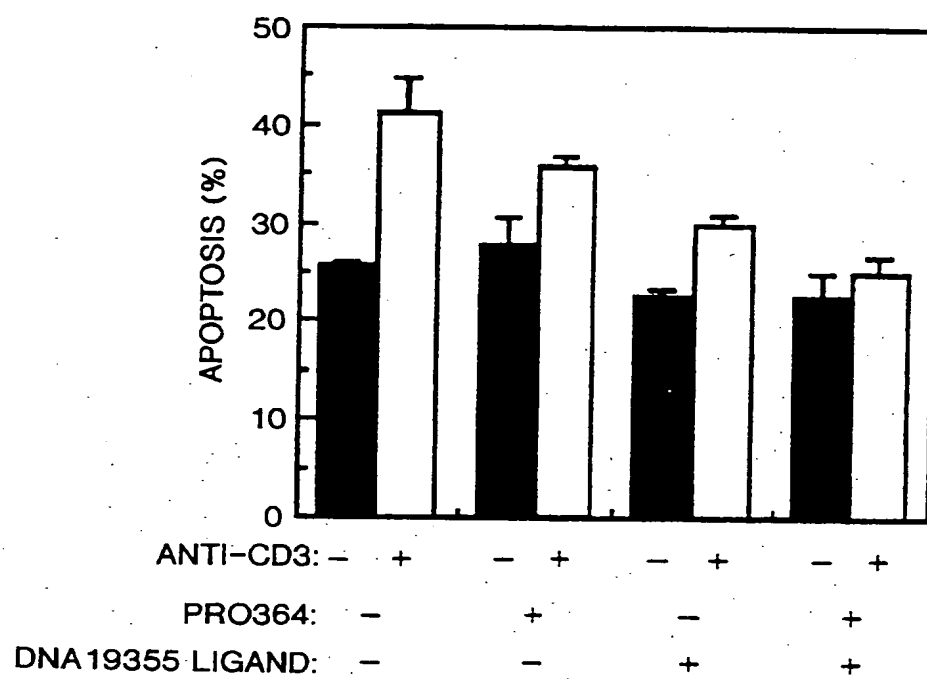


FIG. 13



# INTERNATIONAL SEARCH REPORT

Inter. .onal Application No

PCT/US 99/02642

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C12N5/10 C12N1/19 C12N1/21  
C07K14/705 C07K16/28 C12Q1/68 G01N33/566 A61K38/17  
//C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOCENTINI G. ET AL.: "A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis" PROC. NATL. ACAD. SCI. USA, vol. 94, June 1997, pages 6216-6221, XP002106742 see the whole document ---	1-32
P, X	WO 98 06842 A (SCHERING CORP) 19 February 1998 see abstract see claims 1-19 see seq. ID 4 --- -/--	1-32

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

21 June 1999

Date of mailing of the international search report

06/07/1999

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Fax: (+31-70) 340-3016

Authorized officer

Galli, I

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/02642

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 98 24895 A (RICCARDI CARLO ; PHARMACIA & UPJOHN SPA (IT)) 11 June 1998 see abstract see claims 1-16 see seq. ID 1 ---	1-32
A	US 5-447 851 A (BEUTLER BRUCE A ET AL) 5 September 1995 see abstract ---	23-25
A	ANDERSON D M ET AL: "A HOMOLOGUE OF THE TNF RECEPTOR AND ITS LIGAND ENHANCE T-CELL GROWTH AND DENDRITIC-CELL FUNCTION" NATURE, vol. 390, no. 6656, 13 November 1997, pages 175-179, XP002065548 see the whole document ---	1-32
A	WONG B R ET AL: "FAMILY THAT ACTIVATES C-JUN N-TERMINAL KINASE IN T CELLS. TRANCE IS A NOVEL LIGAND OF THE TUMOR NECROSIS FACTOR RECEPTOR" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 40, 3 October 1997, pages 25190-25194, XP002065547 see the whole document -----	1-32

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 02642

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 30-32  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Nat. Application No

PCT/US 99/02642

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9806842	A	19-02-1998	AU 4055697 A EP 0920505 A	06-03-1998 09-06-1999
WO 9824895	A	11-06-1998	AU 5320798 A	29-06-1998
US 5447851	A	05-09-1995	NONE	